IN THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS

1. (Currently Amended) A method for constructing a mammalian tissue or a fragment thereof in vitro, comprising:

(a) culturing and propagating embryonic epithelial-derived explants, tissue or cells comprising:

(i) isolating the tissues or cells and growing them in culture,

(ii) permitting the tissue or cells to form multiple branches,

(iii) dissecting out individual branch tips,

(iv) culturing the individual branch tips in the presence of nutrient medium, serum, at least one growth factor, and BSN-conditioned medium (BSN-CM) on an extracellular matrix (ECM) gel for several generations to generate branch tip buds;

(b) culturing and propagating isolated embryonic or fetal metanephric mesenchyme comprising:

(i) dissecting out embryonic or fetal metanephric mesenchyme at the time of induction,

(ii) culturing the embryonic or fetal metanephric mesenchymal tissue in the presence of nutrient medium, serum, at least one growth factor, and ~~conditioned medium~~ BSN-CM,

(iii) partitioning mesenchyme into multiple pieces and culturing each piece separately, and

(iv) inducing vasculogenesis by subjecting cultured mesenchyme to substrate deprivation or addition of soluble growth factors;

(c) combining each vascularized mesenchyme with each cultured branch tip bud in a matrix in which in vitro angiogenesis has begun such that the mesenchyme and tip bud are in close contact; and

(d) culturing the combined tissue under conditions to ensure continued ~~vasculogenesis~~ cell growth to obtain a vascularized mammalian tissue,

wherein ~~the~~ at least one growth factor comprises glial cell line-derived neurotrophic factor (GDNF).

2. (Withdrawn) A method for in vitro culturing and propagating ureteric bud tissue, comprising:

isolating embryonic kidney rudiments by dissection,

isolating ureteric bud tissue fragments from mesenchyme by

incubating said kidney rudiments with a proteolytic enzyme in the presence of DNAse and/or by mechanical separation;

suspending said isolated ureteric bud fragments in a gel matrix;

placing the gel/fragment composition on porous polycarbonate membrane inserts in wells of tissue culture plates;

adding growth factors to the culture wells;

maintaining the gel composition at the interface of air and medium until said fragments form multiple tubular branches inside the gel matrix;

dissecting out distal individual branch tips formed during culture; and

reculturing said branched tips in the presence of serum, growth factor mix, cell conditioned medium and nutrient-rich medium for several generations.

3. (Withdrawn) The method according to claim 2, wherein the mechanical separation is accomplished by manual dissection.

4. (Withdrawn) The method according to claim 2, wherein the mechanical separation is accomplished by laser separation and capture.

5. (Previously Presented) The method according to claim 1, wherein the at least one growth factor comprises a glial cell line-derived neurotrophic factor and at least one other growth factor selected from the group consisting of EGF, HGF, IFG, and FGF-2.

6. (Canceled)

7. (Previously Presented) The method according to claim 1, wherein the matrix comprise a mixture of type I collagen and a basement membrane preparation.

8. (Withdrawn) A method for in vitro culturing and propagation of metanephric mesenchyme, comprising:

dissecting out fetal kidney mesenchyme tissue [at the time of induction];

culturing said mesenchymal tissue in the presence of serum, growth factor mix, mesenchymal and/or bud cell conditioned medium and nutrient-rich medium;

partitioning the cultured mesenchyme into multiple pieces and growing each piece separately in culture; and

subjecting grown mesenchyme to substrate deprivation or addition of vasculogenic growth factor sin order to induce vasculogenesis.

9. (Withdrawn) A method for in vitro engineering and constructing a mammalian kidney, comprising:

culturing and propagating a ureteric bud by

isolating the ureteric bud in culture,

permitting the culture to form multiple branches,

dissecting out the individual branch tips,

reculturing in the presence of serum, growth factor mix,

mesenchymal and/or bud cell conditioned medium and nutrient-rich medium for several generations;

culturing and propagating isolated embryonic or fetal metanephric mesenchyme by

dissecting out fetal mesenchyme at the time of induction,

culturing mesenchymal tissue in the presence of serum, growth factor mix,

Gassomuli@aol.com mesenchymal and/or bud cell conditioned medium and nutrient-rich medium,

partitioning the mesenchyme and growing each piece separately, and

inducing vasculogenesis by subjecting grown mesenchyme to substrate

deprivation or addition of vasculogenic growth factors;

recombining each vascularized mesenchyme piece with each cultured bud in a matrix in which in vitro angiogenesis has begun; and

growing in richest medium conditions to ensure continued vasculogenesis.

10. (Currently Amended) The method according to claim 1, wherein ~~the~~ the vascularized mammalian tissue is implanted into a recipient without prior induction of vasculogenesis.

11. (Withdrawn) A function mammalian kidney constructed in vitro from isolated embryonic or fetal kidney tissue or cells that are cultured in rich medium having present a mixture of growth factors and inducer substances, comprising:

an isolated ureteric bud proprgated in culture to produce a functioning nephron;

metanephric mesenchyme propagated from cultured embryonic mesenchymal tissue fragments or cells; and

recombination of propagated ureteric bud and metanephric mesenchyme wherein said recombination in culture results in a functioning kidney or a functionally equivalent fragment thereof.

12. (Previously Presented) The method of claim 1, wherein the vascularized mammalian tissue is mammalian kidney tissue.